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ORIGINAL ARTICLE

High expression of LC3A, LC3B, and p62/SQSTM1 autophagic proteins in human colonic ganglion cells

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Abstract

Introduction: Autophagy is a mechanism that degrades large damaged organelles and misfolded proteins to maintain the homeostasis in all cells. It plays double-faceted roles in tumourigenesis and prevention of various cancers. In our side observation of investigating the prognostic value of autophagy in colorectal cancer (CRC), we found high expression of autophagy proteins (LC3A, LC3B, and p62/SQSTM1) in the colonic ganglion cells. To our best understanding, this is the first paper reporting such finding. *Materials and Methods:* Formalin-fixed paraffin-embedded (FFPE) CRC tissues blocks were retrieved and confirmed by haematoxylin & eosin (H&E) staining. Immunohistochemistry (IHC) targeting autophagy proteins (LC3A, LC3B, and p62/SQSTM1) was then performed followed by pathological examination. *Results:* All three autophagy proteins were present in both normal and tumour tissues of CRC patients. Interestingly, high expression of autophagy proteins in colonic ganglion cells was consistently seen regardless of tissue type (normal or cancer) or tumour site (caecum, ascending, transverse, descending, sigmoid colon and rectum). *Conclusions:* This work highlights the high autophagic activities in human colonic ganglion cells.

Keywords: autophagy; colorectal cancer; colon; ganglion cells; LC3A; LC3B; p62/SQSTM1; immunohistochemistry

INTRODUCTION

Autophagy is a self-digesting defensive mechanism that degrades large damaged organelles and misfolded proteins to maintain the homeostasis in all cells and tissues.[1] Autophagy plays bi-faceted roles in both suppressing and promoting cancer growth.[2] Several decisive factors such as oxidative stress, lipid accumulation and genome instability could contribute to the autophagy-mediated tumour formation or inhibition.[3] In CRC, accumulative evidences demonstrated that autophagy can serve as a therapeutic target[4] and biomarkers for predicting treatment efficacies and prognostication.[5]

In our ongoing project, FFPE CRC tissues blocks were retrieved and IHC targeting the autophagy proteins (LC3A, LC3B, and p62/SQSTM1) was performed. While the CRC tissues showed moderate to strong expression of respective autophagic proteins, consistently high expression of autophagy was also observed in the colonic ganglion cells. In order to search for more information to support or validate this finding, we performed a search on the PubMed library on National Centre for Biotechnology Information (NCBI) website using 'ganglion cells', 'autophagy' and 'colorectal cancer' as keywords. However, none of the reports from the search showed the finding in line with ours which makes our observation worth reporting.

Recent studies have highlighted the role of autophagy in retinal ganglion cells (RGC)[6-8], but not in the colonic ganglion cells. Boya (2017) stressed the important roles of autophagy in axonal protection as well as reducing oxidative

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stress and preserving mitochondrial functions in the retinal ganglion cells. Deficiency of autophagy resulted in the death of retinal ganglion cell and glaucoma.[6] It is known that *Lycium barbarum* or wolfberry increased retinal ganglion cell survival[7] while cigarette smoke extract caused injury in retinal ganglion cells by regulating autophagy.[8] However, autophagy in colonic ganglion cells has never been reported before. In this study, high expression of autophagy protein in human colonic ganglion cells was demonstrated.

MATERIALS AND METHODS

Tissue sample collection

FFPE tissue blocks from CRC patients were collected from the collaborating hospital. This project was approved by Sunway University Research Ethics Committee (SUNREC 2017/051), Sunway Medical Centre Independent Research Ethics Committee (013/2017/ER), and National Medical Research Ethics Committee (NMRR-18-1137-42073).

Immunohistochemistry

FFPE tissue blocks were sectioned at 4μm and placed on a silane-coated slide (MUTO #5116) before heating at 60˚C for at least an hour. Paraffin removal and antigen retrieval were performed using FLEX Target Retrieval Solution Low pH (Dako #K800521) for LC3A and LC3B and High pH (Dako #K800521) for p62/SQSTM1. Slides were heated in a decloaking chamber at 110˚C for 30 minutes and washed in deionized water to remove the retrieval solution residue. The tissues were stained using the REAL Envision Detection System (Dako #K500711) according to the manufacturer's instruction. The tissues were blocked by using the Endogenous Enzyme Block (Dako #S202386) for 10 minutes. The tissues were then incubated overnight in the antibody solution (Table 1) in the humidified slides chamber at 4 ˚C. The tissues were then incubated with the HRP-Polymer for 30 minutes and the DAB+ chromogen was applied for 5–10 minutes. The tissues were then counterstained with hematoxylin and dehydrated with ethanol and cleared with xylene before mounting.

RESULTS

Detectable expression of autophagy proteins (represented by LC3A, LC3B and p62 expression) was found in immunohistochemically stained colorectal cancer tissue sections. Figure 1 shows the cytoplasmic and perinuclear expression of LC3A, LC3B, and p62/SQSTM1 in colorectal adenocarcinoma (malignant glands). Consistent to a previous finding[9], the diffuse and cytoplasmic/ juxta-nuclear staining patterns of autophagy markers were readily observed in the cancerous tissues.

Interestingly, high expression of autophagy proteins was observed in the submucosal and myenteric ganglionic plexus of the colon wall (Fig. 2). The expression was seen in both cancerous and noncancerous tissues. In CRC tissues, we further demonstrate that autophagy expression was consistent across different tissue sites including caecum, ascending, transverse, descending, sigmoid colon and rectum (Fig. 3).

DISCUSSION

Autophagy is an emerging process seen as a key regulator in various diseases including cancers. The importance of this pathway has been recognised and attracted significant attention since the Nobel Prize in Physiology and Medicine 2016 was awarded to Professor Yoshinori Ohsumi who first discovered the pathway. In normal cells, autophagy maintains cellular homeostasis by eliminating damaged organelles and other cytoplasmic molecules through lysosomal degradation.[1] In this brief report, high event of autophagy was observed in human colonic ganglion cells in both cancerous tissues and the adjacent normal cells in CRC.

Ganglion cells are cells within ganglia that provide relay points and intermediary connections between various neurological structures in the body. To date, autophagy has only been related to ganglion cells or neurons located in the inner surface of retina of the eye known as retinal ganglion cells (RGC)[6-8]

Table 1: Details of antibodies used for autophagy expression in FFPE tissues

Antibody	Dilution used	Host species	Clone	Brand
LC3A	1:1,000	Rabbit	D50G8	Cell Signaling Technology
LC3B	1:1,000	Rabbit	D11	Cell Signaling Technology
p62/ SQSTM1	1:500	Rabbit	EPR18351	Abcam

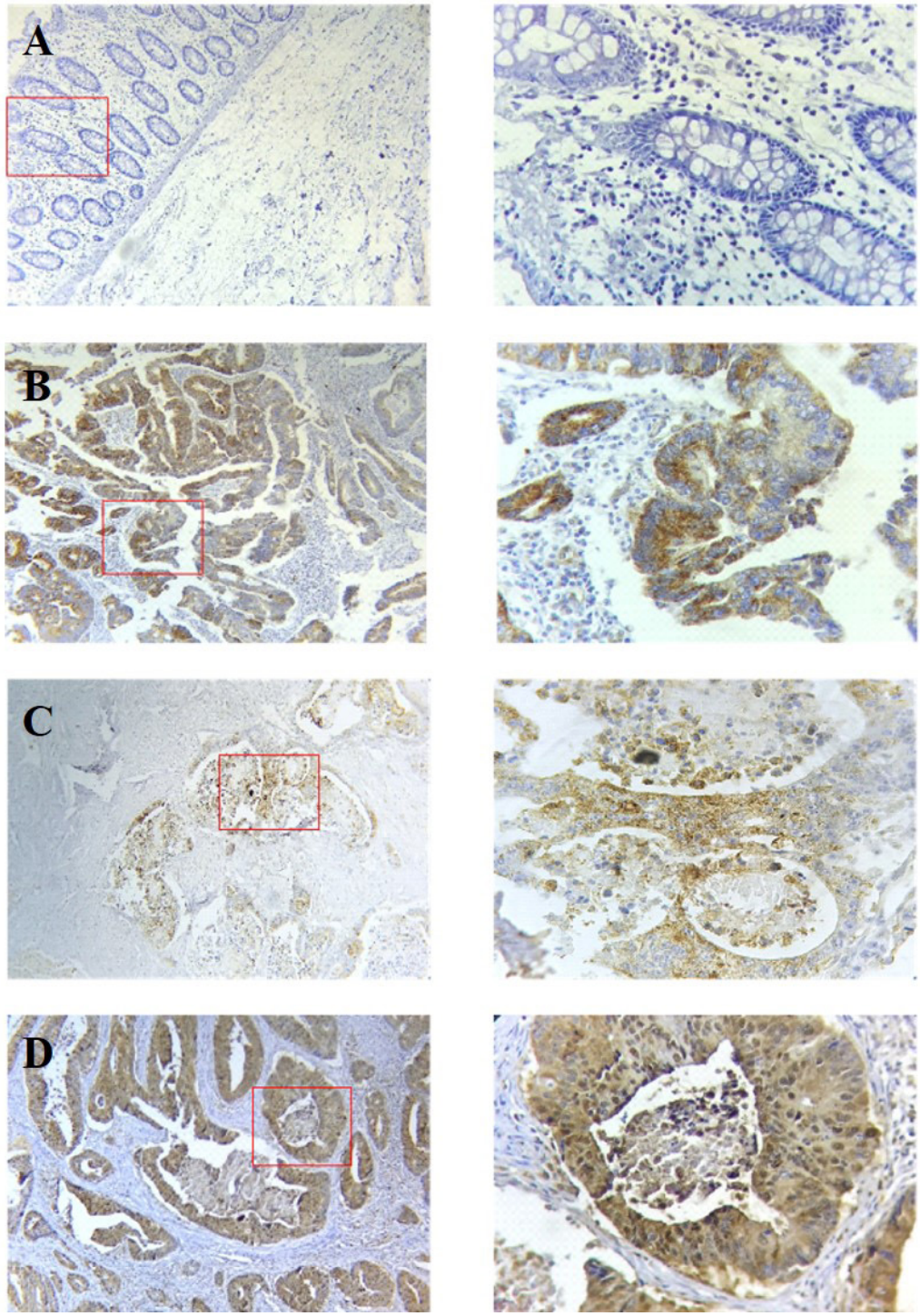

FIG. 1 Cytoplasmic and perinuclear expression of LC3A (B), LC3B (C), and p62/SQSTM1 (D) in colorectal adenocarcinoma (malignant glands) (A – Negative control). The images were from four different cases (magnification x100 on the left panel and corresponding regions highlighted by the red boxes in x400 on the right panel).

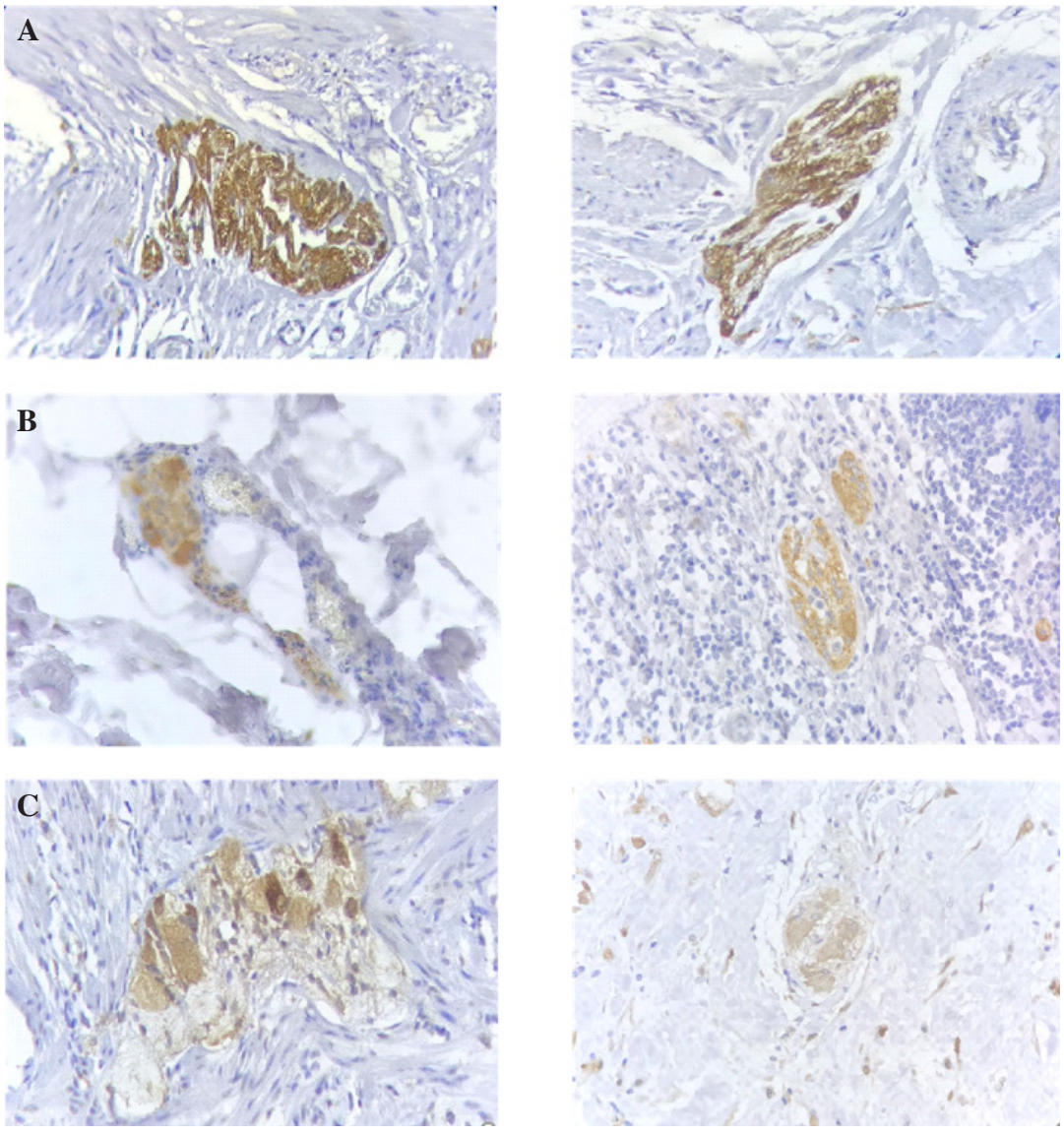

FIG. 2 Expression of LC3A (A), LC3B (B), and p62/SQSTM1 (C) in Meissner's (submucosal) and Auerbach's (Myenteric) ganglionic plexus of the colon wall within normal (left panel) and cancer tissues (right panel) (x400 magnification).

although ganglion cells are present ubiquitously in other parts of the body including the colon. From our findings, we show that autophagy in ganglion cells is less likely associated with the tumourigenesis as moderate to high expression of autophagy proteins were consistently seen in both tumour and normal cells. To further validate this notion, colon tissues from healthy individuals can be tested in parallel to examine the basal expression of autophagy in the ganglion cells, which are lacking in this study.

In ganglion cells particularly RGCs, hypoxia and axonal damage have been shown to induce autophagy.[10,11] As shown by autophagy-deficient mouse models, reduction of autophagy level could lead to diminished protection of cells to axonal stress and damage.[12] Autophagy can eliminate oxidised cellular components such as mitochondria.[13] Reduced level of autophagy has been associated with an increased level of oxidative stress, hence resulting in glaucoma and retinal complications.[13] Autophagy is also linked to ageing in the retina.[14] Due to the lack of study, whether or not autophagy shares the same role in the colonic ganglia such as increased oxidative stress and ageing remains to be seen.

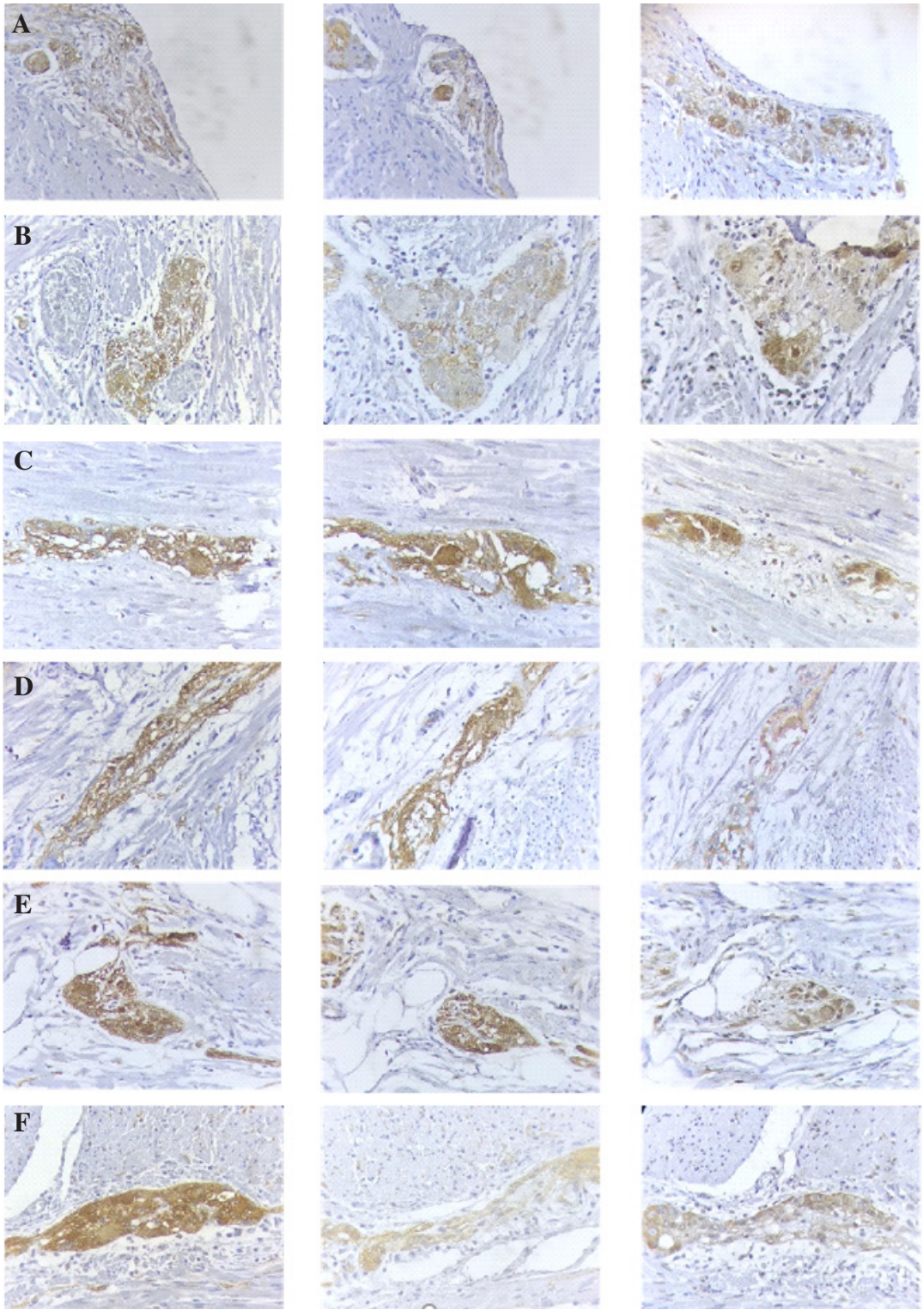

FIG. 3 Expression of LC3A (left panel), LC3B (middle panel), and p62/SQSTM1 (right panel) in ganglion plexus of the colonic wall from different segments of the resected colon cancer tissue (x400 magnification) (A-caecum, B-ascending, C-transverse, D-descending, E-sigmoid colon and F-rectum). Levels from the same tissue block were used for the autophagy protein staining.

In conclusion, the present study shows the autophagy expression in human colonic ganglion cells in both cancerous and normal tissues. To our best knowledge, this is the first study reporting such finding. Further investigations are required to delineate the exact function of autophagy events in the colonic ganglion cells and its implication on other cellular processes.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: This study has previously granted ethical approvals from Sunway Medical Centre Independent Research Ethics Committee (SREC) (013/2017/ER), Sunway University Research Ethics Committee (UREC) (SUNREC 2017/051), and National Medical Research Ethics Committee (MREC) (NMRR-18-1137-42073).

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